CLAIMING

What is claimed is:

1. (Currently amended) The procedure for cloning human βA precursor protein gene (human APP gene) based on the reverse transcription (RT) and the polymerase chain reaction (PCR) using the synthesized oligonucleotides (SEQ ID NO. 1) for RT, and (SEQ ID NO. 2) and (SEQ ID NO. 3) respectively for PCR, comprising:

- Isolating APP-mRNA.
- Performing RT reaction using the synthesized oligonucleotide

  5' GTTACAGCACAG 3' (SEQ ID NO. 1) under the following conditions: 90^0C for 2 minutes; 0^0C for 1 minute; 25^0C for 10 minutes; 42^0C for 45 minutes;
- Performing PCR reaction using the synthesized oligonucleotides

  5' ATGCTGCCCGGTTTGGC 3' (SEQ ID NO. 2) and

  5' CTAGTTCTGCATCTGCTCA 3' (SEQ ID NO. 3) under the following conditions: Denaturing at 94^0C for 1 minutes; annealing at 55^0C for 2 minutes; elongating at 72^0C for 3 minutes each cycle, for 35 cycles;

<u>- Ligating the PCR products of APP gene into the pCR II plasmid vector (SEQ ID NO. 4) and introducing the ligation products in INVαF' E. Coli competent cells;</u>

<u>- Screening for inserts based on the presence of white colonies that results in the selection of the vector (1) (SEQ ID NO. 4 / APP_{751}-cDNA) and the vector (2) (SEQ ID NO. 4 / APP_{770}-cDNA).</u>

2. (Currently amended) The procedure for the construction of expression plasmids using the pFastBac HTb and the pBlueBacHis2 A transfer vectors for the purpose of obtaining human APP in insect cells, comprising:

2.1. Using the pFastBac HTb vector:

- Digesting the pFastBac HTb vector (SEQ ID NO. 5) with Xba I and Hind III followed by dephosphorylation with calf intestinal alkaline phosphatase;

- Digesting the vector (**1**) (SEQ ID NO. 4 / APP_{751}-cDNA) and the vector (**2**) (SEQ ID NO. 4 / APP_{770}-cDNA) with XbaI and HindIII and isolating the resulting fragments containing the cDNA coding sequences of APP, APP_{751}-cDNA and APP_{770}-cDNA;

- Ligating the APP_{751}-cDNA and APP_{770}-cDNA fragments to the pFastBac HTb vector (SEQ ID NO. 5) and introducing the ligation products in INVαF' E. Coli strain;

- Screening for inserts based on the presence of white colonies, as a result of which the vector (**3**) (SEQ ID NO. 5 / APP_{751}-cDNA) and the vector (**4**) (SEQ ID NO. 5 / APP_{770}-cDNA) are selected;

- Introducing the vectors (**3**) and (**4**) in DH10Bac E. Coli competent cells;

- Screening for recombinant bacmids in DH10Bac E. Coli using blue-white color selection, then verifying the presence of APP-cDNA's inserts in the recombinant bacmids by PCR amplification using the M13 forward (-40) and M13 reverse primers, as a result of which the recombinant bacmids (**5**) for vectors (**3**) in DH10Bac E. Coli and (**6**) for vector (**4**) in DH10Bac E. Coli respectively are selected;

2.2. Using the pBlueBacHis2 A vector:

- Digesting the pBlueBacHis2 A vector (SEQ ID NO. 6) with NcoI and HindIII followed by dephosphorylation with calf intestinal phosphatase;

- Digesting the vector (**3**) (SEQ ID NO. 5 / APP_{751}-cDNA) and the vector (**4**) (SEQ ID NO. 5 / APP_{770}-cDNA) with NcoI and HindIII and isolating the resulting fragments containing the cDNA coding sequences of APP, APP_{751}-cDNA and APP_{770}-cDNA;

- Ligating the APP_{751}-cDNA and APP_{770}-cDNA fragments to the pBlueBacHis2 A vector (SEQ ID NO. 6) and introducing the ligation products in INVαF' E. Coli strain;

- Screening for inserts using blue-white color selection, as a result of which the vector (**7**) (SEQ ID NO. 6 / APP_{751}-cDNA) and the vector (**8**) (SEQ ID NO. 6 / APP_{770}-cDNA) are selected.

3. (Currently amended) The procedure for the construction of expression plasmids using the pET-28a (+) transfer vector for the purpose of obtaining human APP in bacteria, comprising:

- Digesting the pET-28a (+) vector (SEQ ID NO. 7) with Sal I and Hind III followed by dephosphorylation with calf intestinal alkaline phosphatase;

- Digesting the vector (**3**) (SEQ ID NO. 5 / APP_{751}-cDNA) and the vector (**4**) (SEQ ID NO. 5 / APP_{770}-cDNA) with Sal I and Hind III and isolating the resulting fragments containing the cDNA coding sequences of APP, APP_{751}-cDNA and APP_{770}-cDNA;

- Ligating the APP_{751}-cDNA and APP_{770}-cDNA fragments to the pET-28a (+) vector (SEQ ID NO. 7) and introducing the ligation products in INVαF' E. Coli strain;

- Screening for inserts based on the presence of white colonies, as a result of which the vector (**9**) (SEQ ID NO. 7 / APP_{751}-cDNA) and the vector (**10**) (SEQ ID NO. 7 / APP_{770}-cDNA) are selected.

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